

AD\_\_\_\_\_

GRANT NUMBER DAMD17-97-1-7303

TITLE: MHC Genes and Breast Cancer

PRINCIPAL INVESTIGATOR: Shiv S. Pillai, M.D., Ph.D.

CONTRACTING ORGANIZATION: Massachusetts General Hospital  
Boston, Massachusetts 02114

REPORT DATE: September 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commanding General  
U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE September 1998		3. REPORT TYPE AND DATES COVERED Annual (15 Aug 97 - 14 Aug 98)
4. TITLE AND SUBTITLE MHC Genes and Breast Cancer			5. FUNDING NUMBERS DAMD17-97-1-7303	
6. AUTHOR(S) Shiv S. Pillai, M.D., Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Massachusetts General Hospital Boston, Massachusetts 02114			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES  <div style="text-align: center; font-size: 2em; font-weight: bold;">1 9 9 9 0 2 2 5 2 0 5</div>				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words)  The goal of this study is to molecularly type all HLA class II genes in patients with breast cancer and in ethnically matched controls, in order to ascertain whether the inheritance of any of these genes contributes to susceptibility or resistance to breast cancer. Detailed molecular typing of DPB, DQB, and DRB1, 3, 4, and 5 alleles was performed on 144 controls. DNA was isolated from 70 freshly selected and cultured lymphoblastoid cell lines derived from patients with breast cancer. Molecular typing of DRB1, 3, 4, and 5 alleles was also performed on these patients. While preliminary results support earlier studies indicating a high frequency of DRB1*0701 in patients with breast cancer, clearly a larger analysis must be completed before attempting to statistically evaluate this information.				
14. SUBJECT TERMS Breast Cancer  HLA Class II genes			15. NUMBER OF PAGES 14	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

## FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

\_\_\_\_ Where copyrighted material is quoted, permission has been obtained to use such material.

\_\_\_\_ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

\_\_\_\_ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

\_\_\_\_ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

SP For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

SP In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

SP In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

\_\_\_\_ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

SP Pillai 9/07/98  
PI - Signature Date

## TABLE OF CONTENTS

	Page number
Cover Page	1
SF298	2
Foreword	3
Table of Contents	4
Introduction, Body, Conclusions	5
Appendices	6-14

## **INTRODUCTION**

This study is based on preliminary results suggesting that the inheritance of the HLA (Human Leukocyte Antigen) class II DRB1\* 0701 allele is linked to breast cancer in women under the age of 40. This data, if supported in a larger and more complete study (involving the genotyping of all HLA class II genes), would lend credence to the notion that immunogenetic factors contribute to the development of breast cancer.

## **BODY**

This application was funded last year, two years after the Preliminary Results were included in the original application (submitted in 1995). Since not all the DNAs from the group of patients examined in our preliminary studies were still available (having been used by the Center for Cancer Risk Analysis at MGH for other studies on the genetics of breast cancer), we resumed most of our studies from scratch. Lymphoblastoid cell lines from a fresh but partially overlapping set of breast cancer patients under the age of 40 were thawed and cultured. DNA was isolated, and totally fresh analyses were performed, even for generic DRB typing. The earlier typing cited in the preliminary results from over 3 years ago, was performed largely by Dr. David Forcione, when he was a medical student working part time in the laboratory. Subsequent typing has been performed over the past year by new personnel who have been trained after the initiation of funding. Given these constraints, we have nonetheless completed a significant portion of the project, starting from scratch.

We have completed a detailed analysis of all DRB1, DRB3, DRB4, DRB5, DPB1 and DQB1 alleles for 144 control subjects. Data from 93 of these has been reviewed and is listed in Tables I through IV. We have completed the analysis of all DRB1, DRB3, DRB4 and DRB5 alleles for 70 patients with breast cancer (only some of whom overlap with our previous 72 patients). Of the 70 breast cancer samples included in our freshly initiated studies we consider data from 3 to be unreliable. These samples are currently being reanalysed along with the next batch of breast cancer DNAs. A complete listing of the individual genotypes of the breast cancer patients is presented in Table V.

## **CONCLUSIONS**

Detailed molecular typing of DPB, DQB, and DRB1, 3, 4, and 5 alleles was performed on 144 controls. DNA was isolated from 70 freshly selected and cultured lymphoblastoid cell lines derived from patients with breast cancer. Molecular typing of DRB1, 3, 4, and 5 alleles was also been performed on these patients. While preliminary results support earlier studies indicating a high frequency of DRB1\*0701 in patients with breast cancer, clearly a larger analysis must be completed before attempting to statistically evaluate this information.

## APPENDICES (TABLES I-V)

**TABLE I**

**DPB1 ALLELES IN CONTROLS (n=93)**

DPB1*0101	7.8% (7)
DPB1*0201	18.9% (17)
DPB1*0202	1.1% (1)
DPB1*0301	20.0% (18)
DPB1*0401	50% (45)
DPB1*0402	30.0% (27)
DPB1*0501	0.0% (0)
DPB1*0601	2.2% (2)
DPB1*0801	1.1% (1)
DPB1*0901	0.0% (0)
DPB1*1001	7.8% (7)
DPB1*1101	2.2% (2)
DPB1*1301	4.4% (4)
DPB1*1401	4.4% (4)
DPB1*1501	3.3% (3)
DPB1*1601	0.0% (0)
DPB1* 1701	2.2% (2)
DPB1*1801	1.1% (1)
DPB1*1901	1.1% (1)

**TABLE I (Continued)**

DPB1*2001	7.8% (7)
DPB1*2101	0.0% (0)
DPB1*2201	0.0% (0)
DPB1*2301	20.0% (18)
DPB1*2401	0.0% (0)
DPB1*2501	1.1% (1)
DPB1*2601	0.0% (0)
DPB1*2701	3.3% (3)
DPB1*2801	0.0% (0)
DPB1*2901	1.1% (1)
DPB1*3001	0.0% (0)
DPB1*3101	0.0% (0)
DPB1*3201	0.0% (0)
DPB1*3301	0.0% (0)
DPB1*3401	0.0% (0)
DPB1*3501	2.2% (2)
DPB1*3601	1.1% (1)

**TABLE II****DQB1 ALLELES IN CONTROLS (n=86)**

DQB1*0201	24.4% (21)
DQB1*0301	46.5% (40)
DQB1*0302	19.8% (17)
DQB1*03031	0.0% (0)
DQB1*03032	9.3% (8)
DQB1*0401	0.0% (0)
DQB1*0402	4.7% (4)
DQB1*0501	18.6% (16)
DQB1*0502	4.7% (4)
DQB1*05031	9.3% (8)
DQB1*05032	0.0% (0)
DQB1*0504	0.0% (0)
DQB1*0601	1.2% (1)
DQB1*0602	26.7% (23)
DQB1*0603	11.6% (10)
DQB1*0604	4.7% (4)
DQB1*0605	0.0% (0)



**TABLE III****DRB1 ALLELES IN CONTROLS (n=93)**

DR1	*0101	10.8% (10)
	*0102	6.5% (6)
	*0103	4.3% (4)
DR2	*1501	25.8% (24)
	*1502	1.1%(1)
	*1503	0.0% (0)
	*1601	4.3% (4)
	*1602	0.0% (0)
DR3	* 0301-02	17.2% (16)
DR4	*0401	12.9% (12)
	*0402	6.5% (6)
	*0403	2.2% (2)
	*0404	5.4% (5)
	*0405	0.0% (0)
	*0406	0.0% (0)
	*0407	5.4% (5)
	*0408	1.1% (1)
	*0409	0.0% (0)
	*0410	0.0% (0)

	*0411	0.0% (0)
DR7	*0701	16.1% (15)
DR8	*0801-04	4.3% (4)
DR9	*0901A/B	1.1% (1)
DR10	*1001	1.1% (1)
DR11	*1101	15.0% (14)
	*1102	1.1% (1)
	*1103	2.2% (2)
	*1104	8.6% (8)
DR12	*1201-02	5.4% (5)
DR13	*1301	10.8% (10)
	*1302	5.4% (5)
	*1303	1.1% (1)
	*1304	0.0% (0)
	*1305	0.0% (0)
DR14	*1401	9.7% (9)
	*1402	0.0%

\*1403 1.1% (1)  
\*1404 2.2% (2)  
\*1405 0.0% (0)  
\*1406 0.0% (0)  
\*1407 0.0% (0)  
\*1408 0.0% (0)

**TABLE IV**  
**DRB3, DRB4 and DRB5 ALLELES IN CONTROLS (n=93)**

**DRB3**

*0101	23.7% (22)
*0201/*0202	46.2% (43)
*0301	5.4% (5)

**DRB4**

*0101	45.2% (42)
-------	------------

**DRB5**

*0101	28.0% (26)
*0102/*02	3.2% (3)

TABLE V	I.D.#	MHC DRB1	MHC DRB1	MHC DRB3/4/5	MHC DRB3/4/5
		allele	allele	allele	allele
GENOTYPING OF BREAST CANCER SUBJECTS	Brl 04	* 1601	* 0102	DRB4 * 0101	DRB5 * 0101
	Brl 10	* 0402	—	DRB4 * 0101	DRB3 * 02
	Brl 08	* 14	* 04	DRB3 * 0202	DRB4 * 0101
	Brl 11	* 08	—	DRB3 * 0202	DRB4 * 0101
	Brl 12	* 0302	* 15	DRB3 * 0101	DRB5 * 0101
	Brl 13	* 1501	—	DRB3 * 0202	DRB4 * 0101
	Brl 15	* 0103	* 15	DRB3 * 0202	DRB5 * 0101
	Brl 17	* 0103	* 0103	DRB3 * 0202	DRB4 * 0101
	Brl 18	* 0302	—	—	—
	Brl 30	* 1403	—	DRB3 * 0202	DRB4 * 0101
	Brl 34	* 07	* 1403	DRB3 * 0202	DRB4 * 0101
	Brl 36	* 1302	* 1501	DRB3 * 0101	DRB5
	Brl 37	* 0302	—	DRB4 * 0101	
	Brl 39	* 0402	* 0302	DRB3 * 0101	DRB4 * 0101
	Brl 42	* 07	* 1403	DRB3 * 0202	DRB4 * 0101
	Brl 47	* 0101	* 07	DRB4 * 0101	
	Brl 52	* 0401	* 1302	DRB3 * 0301	DRB4 * 0101
	Brl 54	* 1501	* 0401	DRB4 * 0101	
	Brl 55	* 1501	* 0401	DRB3 * 0202	DRB4 * 0101
	Brl 56	* 1303	* 1303	DRB3 * 0101	DRB3 * 0101
	Brl 57	* 0102	* 0302	DRB3 * 0101	DRB4
	Brl 61	* 04	—	DRB3 * 0202	DRB4 * 0101
	Brl 63	* 1302	* 0302	DRB3 * 02	
	Brl 65	* 0401	* 1302	DRB3 * 0202	DRB4 * 0101
	Brl 68	* 0401	* 0302	DRB3 * 0202	DRB4 * 0101
	Brl 70	* 0401	* 14	DRB3 * 0202	DRB4 * 0101
	Brl 71	* 07	* 1202	DRB3 * 0202	DRB4 * 0101
	Brl 72	—	—	DRB4 * 0101	
	Brl 74	* 1501	* 0407	DRB3 * 0202	DRB5 * 0101
	Brl 75	* 04	* 1602	DRB3 * 0202	DRB4 * 0101
	Brl 77	* 0302	—	DRB3 * 0202	DRB5 * 0101
	Brl 78	—	—	DRB3 * 0202	DRB5 * 0101
	Brl 81	* 0101	* 07	DRB3 * 0202	DRB4 * 0101
	Brl 83	* 04	* 0302	DRB3 * 0101	DRB4 * 0101
	Brl 85	* 0404	* 1403	DRB3 * 0202	DRB4 * 0101
	Brl 88	* 07	* 1503	DRB3 * 0201	DRB5 * 0101
	Brl 89	* 1502	—	DRB3 * 0201	DRB5 * 0101
	Brl 90	* 1403	—	DRB3 * 0202	DRB5 * 01
	Brl 93	* 07	* 1403	DRB3 * 0202	—
	Brl 95	* 0405	* 1302	DRB3 * 0101	—
	Brl 97	* 07	—	DRB3 * 0201	—
	Brl 98	* 1303	—	DRB3 * 0101	—
	Brl 99	* 1302	—	DRB3 * 0301	—

I.D.#	MHC DRB1 allele	MHC DRB1 allele	MHC DRB3/4/5 allele	MHC DRB3/4/5 allele
Brl 101	* 07	*0407	DRB3 * 0202	—
Brl 102	* 0302	* 1601	DRB3 * 0202	—
Brl 103	* 0408	*1501	DRB3 * 0202	DRB5
Brl 104	* 07	* 0101	DRB3 * 0202	—
Brl 106	* 0407	* 0302	DRB3 * 0101	—
Brl 108	* 1303	—	DRB3 * 0101	—
Brl 109	* 07	* 1403	DRB3 * 0202	—
Brl 111	* 1503	—	DRB3 * 1010	DRB5
Brl 112	* 07	* 1501	DRB3 * 0202	DRB5
Brl 118	* 1501	* 1302	DRB3 * 0101	—
Brl 119	* 0404	* 1403	DRB3 * 0202	—
Brl 121	* 07	—	DRB3 * 0202	—
Brl 122	* 0302	—	DRB3 * 0101	—
Brl 123	* 0407	* 1302	DRB3 * 0301	—
Brl 124	* 0302	—	DRB3 * 0101	—
Brl 125	* 0302	*0408	DRB3 * 0301	—
Brl 126	* 1501	* 0101	DRB3 * 0202	—
Brl 128	* 0101	* 1501	DRB3 * 0202	—
Brl 129	* 1501	* 0408	DRB3 * 0202	—
Brl 132	* 0302	—	DRB3 * 0101	—
Brl 133	* 1501	* 1202	DRB3 * 0202	—
Brl 137	* 1401	—	DRB3 * 0202	—
Brl 139	* 1501	—	DRB3 * 0202	DRB5
Brl 140	* 1501	* 0402	DRB3 * 0201	—